

## Product Information

### CellView Probes for Long-Term Tracing of Living Cells

Catalog Number	Product Name	Unit Size
C037	CellView Blue CMAC	5 mg
C039	CellView Green CMFDA	1 mg
C040	CellView Orange CMTMR	1 mg

#### Storage upon receipt:

- 20°C
- Protect from light

### Product Description

Applied BioProbes has developed a series of CellView fluorescent probes that are retained in living cells through several generations. The probes are inherited by daughter cells after cell fusion and are not transferred to adjacent cells in a population. These CellView reagents can be loaded into cells by adding the reagent to the culture medium and then washing the cells briefly with fresh medium before analysis. These reagents pass freely through cell membranes, but once inside the cell, are transformed into cell-impermeant reaction products. The CellView dyes contain a chloromethyl group that reacts with thiols, probably in a glutathione S-transferase-mediated reaction, since this has been shown to occur *in vitro*<sup>1</sup>. In most cells, glutathione levels are high (up to 10 mM) and glutathione transferase is ubiquitous. The reagent is transformed into a cell-impermeant fluorescent dye-thioether adduct that can be fixed with aldehyde fixatives, permitting long-term sample storage. Excess unconjugated reagent passively diffuses to the extracellular medium.

Fluorescent CellView reagents include the blue-fluorescent chloromethyl derivative of aminocoumarin (CMAC), the greenfluorescent chloromethyl derivative of fluorescein diacetate (CMFDA), and the orange-fluorescent CMTMR. The blue (CMAC), green (CMFDA), and orange (CMTMR) fluorescence in the cells is reasonably photostable during microscopic examination. Cells stained with CMAC, CMFDA and CMTMR probes were brightly fluorescent for at least 72 hours after incubation in fresh medium at 37°C and through at least four cell divisions (retention time of these probes within the cell can vary dependent upon the cell type, incubation conditions and other factors and should be empirically determined prior to any tracing experiments). No other permeant dyes of this type, including the widely used calcein AM and BCECF-AM, are retained in viable cells for more than a few hours at such physiological temperatures.

The CellView reagents represent a major breakthrough in the cellular retention of vital probes and are excellent tools for long-term studies of normal and transformed cells in culture and for investigating cellular thiol levels, cell viability and cytotoxicity, transplantation and cell fusion.

The following protocol describes introducing the CellView reagent into the cultured cells and imaging the stained cells by fluorescence microscopy. Various factors, such as penetration of the dye into the cells or tissue, may require that some conditions be modified for particular cell types.

The optimal concentration of the probe for staining varies depending upon the application. Testing at least a tenfold range of concentrations is recommended. In general, long-term staining (more than about 3 days) or the use of rapidly dividing cells requires 5–25 µM dye. Less dye (0.5–5 µM) is needed for shorter experiments, such as viability assays. To maintain normal cellular physiology and reduce potential artifacts, keep the dye concentration as low as possible.

#### Staining Protocol

**1.1** Dissolve the lyophilized product in high-quality DMSO to a final concentration of 10 mM. Dilute the stock solution to a final working concentration of 0.5–25 µM in serum-free medium. Warm the working solution to 37°C.

**1.2** For cells in suspension, harvest cells by centrifugation and aspirate the supernatant. Resuspend the cells gently in prewarmed CellView dye working solution. Incubate cells for 15–45 minutes under growth conditions appropriate for the particular cell type. Centrifuge the cells. For adherent cells, when the cells have reached the desired confluence, remove the medium from the dish and add the prewarmed CellView dye working solution. Incubate the cells for 15–45 minutes under growth conditions appropriate for the particular cell type.

**1.3** Replace the dye working solution with fresh, prewarmed medium and incubate the cells for another 30 minutes at 37°C. During this time, the chloromethyl group (and for some probes, the acetate group) of the dye undergoes modification or are secreted from the cell.

**1.4** Attach suspended cells to coverslips.

**1.5** Wash cells with PBS.

**1.6** Fix the cells with 3.7% formaldehyde in PBS for 15 minutes at room temperature, if desired.

**1.7** Wash cells with PBS.

**1.8** Permeabilize the cells, if desired. When the cells are going to be subsequently labeled with an antibody, a permeabilization step is often required to enhance the antigen's accessibility. Permeabilize cells by incubating cells in ice-cold acetone for 10 minutes.

**Table.** Spectral characteristics of the CellView probes

Product Name	Ex (nm)	Em (nm)
CellView Blue CMAC	353	466
CellView Green CMFDA	492	517
CellView Orange CMTMR	541	565

### Experimental Protocols